BRD4 Structure–Activity Relationships of Dual PLK1 Kinase–BRD4 Bromodomain Inhibitor BI-2536

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SUPPORTING INFORMATION

Chemistry

All reactions were performed in oven-dried glassware under and inert (N_2) atmosphere, unless otherwise stated. Anhydrous solvents were used as supplied without further purification. ^1H and ^{13}C NMR spectra were recorded on a Varian 400 MHz NMR spectrometer at 25 °C. Chemical shifts are reported in parts per million (ppm) and are referenced to residual non-deuterated solvent peak ($CHCl_3$: δ_H, 7.26 δ_C 77.2; DMSO: δ_H 2.50, δ_C 39.5). Mass spectra were recorded on a Bruker AmaZon X mass spectrometer using atmospheric pressure chemical ionization (APCI). All final molecules were confirmed to be >90% pure by HPLC prior to biological testing using a Waters 1525 analytical/preparative HPLC equipped with a Atlantis T3 C18 reversed phase column according to a gradient of 50% solvent (A) to 100% solvent (B) over 10 min at 1 ml min^{-1}, where solvent (A) is H_2O with 0.1% TFA and (B) is CH_3CN-H_2O, 9:1 with 0.1% TFA.

General procedure A: Methyl ester synthesis. Compound **2**, **3**, **4** or **5** (1 eq) was suspended in methanol (0.1 M), and $SOCl_2$ (2 eq) was added slowly at 0 °C, then the reaction was heated at reflux. After 1.5 h, TLC indicated the reaction was complete. The volatiles were evaporated and the residue was triturated with Et_2O. The resulting white solid was filtered and dried under vacuum, yielding the methyl ester as its HCl salt.

General procedure B: Reductive amination. The methyl ester HCl salt (1 eq) and appropriate ketone or aldehyde (1 eq) were dissolved in 1,2-dichloroethane (0.1 M), followed by NaOAc (1 eq) and $NaBH(OAc)_3$ (1.5 eq) at 0 °C. The reaction mixture was stirred at RT overnight. TLC indicated the reaction was finished. To quench the reaction, saturated $NaHCO_3$ solution was added, and then the reaction mixture was partitioned with CH_2Cl_2. The organic layer was collected and then the aqueous layer was extracted with CH_2Cl_2 (x2). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated to yield compounds **6–11**.

General procedure C: Nucleophilic aromatic substitution (S_NAr). Compound **6**, **7**, **8**, **9**, **10** or **11** (1 eq) was dissolved in acetone (0.1 M), then and K_2CO_3 (1 eq) was added, followed by 2,4-dichloro-5-nitropyrimidine (1.06 eq) at 0 °C. The reaction was stirred at RT overnight. TLC indicated the reaction was complete. The acetone was removed *in vacuo*, and then the residue was re-dissolved in EtOAc, and washed with water. The organic layer was collected and dried over Na_2SO_4, filtered, concentrated, and purified by flash column chromatography over silica gel using an eluent of Hex/EtOAc 9:1 to give compounds **12–17**.

General procedure D: Reduction of nitro group and heterocyclization. Compound **12**, **13**, **14**, **15**, **16** or **17** (1 eq) was dissolved in glacial acetic acid (0.4 M) and heated to 70 °C. Iron powder (1.2 eq) was added portionwise over a few minutes. The reaction was stirred for 1 h at 70 °C then 4-5 h at 100 °C until complete (TLC). The mixture was filtered through Celite, rinsing with methanol. The volatiles were evaporated, and then the residue was purified by flash column chromatography over silica gel using a gradient of Hex/EtOAc 8:2 to 6:4 to yield compounds **18–23**.

General procedure E: Alkylation. Compound **18**, **19**, **20**, **21**, **22** or **23** (1 eq) was dissolved in anhydrous DMF (0.1 M), followed by the requisite alkyl iodide or bromide (1.3 eq). The mixture was cooled to -10 °C, then NaH (1.3 eq) was added. The reaction was stirred at RT for 3 h unless otherwise stated. TLC indicated the reaction was complete. Ice was added to the reaction mixture to quench the reaction. The reaction mixture was partitioned between EtOAc/H_2O, and the organic layer was collected. The aqueous layer was extracted with further EtOAc. The combined organic layers were washed with water (x2), dried over Na_2SO_4, filtered, concentrated then purified by

flash column chromatography over silica gel using a gradient of Hex/EtOAc 4:1 to yield compounds **24–33**.

General procedure F: Amide synthesis. One of compounds **34a–34g** (1 eq) was dissolved in anhydrous DMF (0.1 M), followed by HBTU (1.6 eq), 4-amino-1-methylpiperidine (1 eq) and triethylamine (2 eq). The reaction mixture was stirred at RT overnight. TLC indicated that the reaction was finished. The reaction mixture was partitioned between EtOAc and water. The EtOAc layer was collected, and the aqueous layer was extracted twice more with EtOAc. The organic separations were pooled, washed with 0.1 M NaOH (x5), and then collected, dried over Na_2SO_4, filtered and concentrated to yield products **35a–35g**.

General procedure G: Reduction of nitro group with $SnCl_2{\cdot}2H_2O$. One of compounds **35a–35g** (1 eq) was dissolved in a solvent mixture of EtOAc/EtOH 10:3 (0.1 M), followed by $SnCl_2{\cdot}2H_2O$ (5 eq). The reaction was stirred at 50 °C overnight. TLC indicated the reaction was complete. The reaction mixture was allowed to cool, then saturated $NaHCO_3$ was added to quench the reaction. The reaction mixture was partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was collected, and the aqueous layer was extracted with further EtOAc (x2). The organic layers were collected, combined, dried over Na_2SO_4, filtered and concentrated to furnish compounds **36a–36g**.

General procedure H: Nucleophilic aromatic substitution (S_NAr). One of compounds **24–33** (1 eq) and one of compounds **36a–36g** or **38** (1 eq) were dissolved in a solvent mixture of EtOH/H_2O/dioxane 1:1:1 (0.1 M). Concentrated hydrochloric acid (2.1 eq) was added. The reaction mixture was refluxed until TLC indicated that the reaction was complete (typically, 24 – 48 h). The mixture was partitioned between EtOAc and NaOH (1 M). The organic layer was collected, dried over Na_2SO_4, filtered, concentrated, and purified by preparative TLC using a solvent mixture of CH_2Cl_2/MeOH/NH_4OH, 92:7:1 to yield compounds **1, 39a–39p**.

(R)-Methyl-2-(cyclopentylamino)butanoate **(6).**[23] D-2-Aminobutyric acid **(2)** was converted to its methyl ester according to general procedure A on a 19.4 mmol scale, then the product was coupled to cyclopentanone according to general procedure B to provide compound **6** as a light yellow oil (3.41g, 95%): ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.69 (3H, s, CH_3), 3.19 (1H, t, *J* = 7.2Hz, NH), 2.94 (1H, q, *J* = 6.4Hz, CH), 1.79-1.58 (6H, m, Cp), 1.52-1.46 (2H, m, Cp), 1.28 (2H, qn, *J* = 5.6Hz, CH_2), 0.88 (3H, t, *J* = 6.8Hz, CH_3).

Methyl-2-(cyclopentylamino)acetate **(7).** Glycine **(3)** was converted to its methyl ester according to general procedure A on an 8 mmol scale, then the product was coupled to cyclopentanone according to general procedure B to deliver compound **7** as a light yellow oil (1.31g, 86%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 3.62 (3H, s, CH_3), 3.02-3.00 (1H, m, CH), 1.68-1.27 (8H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 172.9, 172.5, 63.9, 58.8, 51.7, 49.8, 49.1, 32.6, 29.6, 23.9, 23.7, 21.5.

(S)-Methyl 2-(cyclopentylamino)butanoate **(8).** L-2-Aminobutyric acid **(4)** was converted to its methyl ester according to general procedure A on a 10 mmol scale, then the product (7.1 mmol) was coupled to cyclopentanone according to general procedure B to provide compound **8** as a light yellow oil (1.3 g, 98%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 3.64 (3H, s, O-CH_3), 3.10 (1H, t, *J* = 6.4Hz, C<u>H</u>CH_2CH_3), 2.93-2.88 (1H, m, CH(Cp)), 1.69-1.24 (10H, m, Cp and C<u>H</u>$_2CH_3$), 0.85 (3H, t, *J* = 8.0Hz, CH_2C<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 176.2, 61.3, 57.8, 51.6, 33.6, 32.5, 26.7, 23.8, 23.7, 10.7.

(R)-Methyl-2-(cyclopentylamino)-3-phenylpropanoate **(9).** D-Phenylalanine **(5)** was converted to its methyl ester according to general procedure A on a 10 mmol scale, then the product (7 mmol) was coupled to cyclopentanone according to general procedure B to provide compound **9** as a light yellow oil (1.6 g, 95%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.27-7.23 (2H, m, Ar), 7.20-7.15 (3H, m, Ar), 3.52 (3H, s, O-CH_3), 3.43 (1H, t, *J* = 7.2Hz, C<u>H</u>CH_2Ph), 2.94-2.89 (1H, m, CH(Cp)), 2.85-2.75 (2H, m, C<u>H</u>$_2$Ph), 1.99 (1H, s, NH), 1.67-1.19 (8H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 175.4, 138.3, 129.4, 128.5, 126.7, 61.8, 57.7, 51.6, 33.6, 32.4, 23.8, 23.7.

(R)-Methyl-2-(isobutylamino)butanoate **(10).** D-2-Aminobutyric acid **(2)** was converted to its methyl ester according to general procedure A on a 19.4 mmol scale, then the product (6.5 mmol) was coupled to isobutyraldehyde according to general procedure B to provide compound **10** as a light yellow oil (1 g, 95%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 3.62 (3H, s, O-CH_3), 3.06 (1H, t, *J* = 6.4Hz, C<u>H</u>CH_2CH_3), 2.31-2.26 (1H, m, N-C<u>H</u>$_a$CH), 2.17-2.14 (1H, m, N-C<u>H</u>$_b$CH), 1.60-1.51 (3H, m, C<u>H</u>$(CH_3)_2$ and C<u>H</u>$_2CH_3$), 0.87-0.82 (9H, m, CH(C<u>H</u>$_3)_2$ and CH_2C<u>H</u>$_3$).

(R)-Methyl-2-((3-bromobenzyl)amino)butanoate **(11).** D-2-Aminobutyric acid **(2)** was converted to its methyl ester according to general procedure A on a 19.4 mmol scale, then the product (6.5 mmol) was coupled to 3-bromobenzaldehyde according to general procedure B to provide compound **11** as a light yellow oil (1.6 g, 86%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.48 (1H, s, Ar), 7.37 (1H, d, *J* = 7.6Hz, Ar), 7.27-7.20 (2H, m, Ar), 3.70 (1H, d, *J* = 14.0Hz, C<u>H</u>$_a$Ph), 3.58 (3H, s, O-CH_3), 3.52 (1H, d, *J* = 14.0Hz, C<u>H</u>$_b$Ph), 3.04 (1H, m, C<u>H</u>CH_2CH_3), 1.56-1.52 (2H, m, CHC<u>H</u>$_2CH_3$), 0.82 (3H, t, *J* = 4.0Hz, $CHCH_2$C<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 175.4, 143.9, 130.9, 130.7, 129.8, 127.3, 121.9, 61.9, 51.7, 50.6, 26.2, 10.7.

(R)-Methyl-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)butanoate **(12).**[23] Compound **6** was coupled to 2,4-dichloro-5-nitropyrimidine according to general procedure C on a 5.4 mmol scale to yield compound **12** as a yellow powder (756 mg, 40%): ^{1}H NMR ($CDCl_3$, 400MHz) δ 8.64 (1H, s, pyrimidine), 3.73 (3H, s, O-CH_3), 3.72-3.70 (1H, m, C<u>H</u>CH_2CH_3), 3.53-3.51 (1H, m,

CH(Cp)), 2.42-2.35 (1H, m, CHC$\underline{H}_a$CH$_3$), 2.06-1.95 (1H, m, CHC$\underline{H}_b$CH$_3$), 1.80-1.46 (8H, m, Cp), 1.02 (3H, t, *J* = 7.2Hz, CH$_2$C$\underline{H}_3$).

Methyl-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)acetate **(13).** Compound **7** was coupled to 2,4-dichloro-5-nitropyrimidine according to general procedure C on a 6.7 mmol scale to yield compound **13** as a dark brown powder (682 mg, 35%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.64 (1H, s, pyrimidine), 4.16 (2H, s, CH$_2$), 4.00-3.90 (1H, m, CH), 3.78 (3H, s, CH$_3$), 2.20-2.06 (2H, m, Cp), 1.72-1.53 (6H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 168.8, 160.2, 156.3, 154.8, 131.1, 62.2, 52.6, 46.7, 28.9, 23.9.

(S)-Methyl-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)butanoate **(14).** Compound **8** was coupled to 2,4-dichloro-5-nitropyrimidine according to general procedure C on a 4.9 mmol scale to yield compound **14** as a yellow powder (1 g, 59%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.83 (1H, s, pyrimidine), 4.22 (1H, t, *J* = 7.2Hz, C$\underline{H}$CH$_2$CH$_3$), 3.63 (3H, s, CH$_3$), 3.52-3.47 (1H, m, CH(Cp)), 2.25-2.18 (1H, m, CHC$\underline{H}_a$CH$_3$), 2.00-1.42 (9H, m, Cp and CHC$\underline{H}_b$CH$_3$), 0.94 (3H, t, *J* = 7.2Hz, CH$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 171.2, 158.4, 157.8, 154.2, 131.4, 64.1, 59.3, 52.4, 29.8, 26.8, 22.8, 22.7, 22.5, 11.4.

(R)-Methyl-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)-3-phenylpropanoate **(15).** Compound **9** was coupled to 2,4-dichloro-5-nitropyrimidine according to general procedure C on a 4.7 mmol scale to yield compound **15** as a yellow powder (350 mg, 17%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.87 (1H, s, pyrimidine), 7.25-7.21 (2H, m, Ar), 7.18-7.15 (3H, m, Ar), 4.51-4.47 (1H, m, C$\underline{H}$CH$_2$Ph), 3.67 (3H, s, CH$_3$), 3.49-3.44 (1H, m, CH(Cp)), 3.29-3.23 (2H, m, C$\underline{H}_2$Ph), 1.73-1.06 (8H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 170.7, 158.4, 157.8, 154.3, 137.9, 131.5, 130.5, 128.5, 127.1, 64.1, 59.9, 52.6, 34.5, 29.9, 25.2, 25.5, 22.4.

(R)-Methyl-2-((2-chloro-5-nitropyrimidin-4-yl)(isobutyl)amino)butanoate **(16).** Compound **10** was coupled to 2,4-dichloro-5-nitropyrimidine according to general procedure C on a 3.4 mmol scale to yield compound **16** as a yellow powder (796 mg, 70%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.84 (1H, s, pyrimidine), 4.36 (1H, t, *J* = 7.6Hz, C$\underline{H}$CH$_2$CH$_3$), 3.61 (3H, s, O-CH$_3$), 3.18-3.13 (1H, m, C$\underline{H}_a$CH), 3.03-2.97 (1H, m, C$\underline{H}_b$CH), 2.04-1.87 (3H, m, C$\underline{H}$(CH$_3$)$_2$ and CHC$\underline{H}_2$CH$_3$), 0.91 (3H, t, *J* = 6.8Hz, CH$_2$C$\underline{H}_3$), 0.79 (3H, d, *J* = 6.8Hz, CH(C$\underline{H}_3$)$_2$), 0.68 (3H, d, *J* = 6.8Hz, CH(C$\underline{H}_3$)$_2$).

(R)-Methyl-2-((3-bromobenzyl)(2-chloro-5-nitropyrimidin-4-yl)amino)butanoate **(17).** Compound **11** was coupled to 2,4-dichloro-5-nitropyrimidine according to general procedure C on a 5.7 mmol scale to yield compound **17** as a yellow powder (1.8 g, 73%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.64 (1H, s, pyrimidine), 7.45-7.40 (2H, m, Ar), 7.27-7.21 (1H, m, Ar), 7.15 (1H, t, *J* = 7.2Hz, Ar), 4.75-4.68 (2H, m, C$\underline{H}$CH$_2$CH$_3$ and C$\underline{H}_a$Ph), 4.58 (1H, d, *J* = 15.6Hz, C$\underline{H}_b$Ph), 3.81 (3H, s, CH$_3$), 2.28-2.23 (1H, m, CHC$\underline{H}_a$CH$_3$), 2.09-2.02 (1H, m ,CHC$\underline{H}_b$CH$_3$), 1.06 (3H, t, *J* = 6.8Hz, CH$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 194.4, 170.7, 156.7, 155.3, 135.7, 131.8, 131.4, 130.2, 127.2, 122.7, 64.9, 52.8, 52.6, 23.5, 11.1.

(R)-2-Chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one **(18).**[23] Compound **12** was reductively heterocyclized on a 2.2 mmol scale with iron powder and acetic acid according to general procedure D to yield compound **18** as a yellow powder (247 mg, 40%): ^{1}H NMR ($CDCl_3$, 400MHz) δ 8.83 (1H, s, NH), 7.70 (1H, s, pyrimidine), 4.32-4.30 (1H, m, CH(Cp)), 4.20-4.19 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 2.06-1.77(8H, m, Cp), 1.69-1.54 (2H, m, CHC$\underline{H}_2$CH$_3$), 0.92 (3H, t, *J* = 6.8Hz, CH$_3$).

2-Chloro-8-cyclopentyl-7,8-dihydropteridin-6(5H)-one **(19).** Compound **13** was reductively heterocyclized on a 2.1 mmol scale with iron powder and acetic acid according to general procedure D to yield compound **19** as a yellow powder (280 mg, 53%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.77 (1H, s, NH), 7.52 (1H, s, pyrimidine), 4.92-4.83 (1H, m, CH(Cp)), 4.05 (2H, s, CH$_2$), 1.77-1.55 (8H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 163.1, 152.5, 151.7, 137.9, 119.8, 55.2, 45.2, 26.9, 24.2.

(S)-2-Chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one **(20).** Compound **14** was reductively heterocyclized on a 1.2 mmol scale with iron powder and acetic acid according to general procedure D to yield compound **20** as a yellow powder (265 mg, 82%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.85 (1H, s, NH), 7.56 (1H, s, pyrimidine), 4.23-4.22 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.13-4.09 (1H, m, CH(Cp)), 1.93-1.53 (10H, m, Cp and CHC$\underline{H}_2$CH$_3$), 0.77 (3H, t, *J* = 7.2Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.7, 152.2, 151.6, 138.3, 119.7, 61.5, 59.8, 28.7, 26.8, 24.3, 8.8.

(R)-7-Benzyl-2-chloro-8-cyclopentyl-7,8-dihydropteridin-6(5H)-one **(21).** Compound **15** was reductively heterocyclized on a 0.79 mmol scale with iron powder and acetic acid according to general procedure D to yield compound **21** as a pale yellow foam (180 mg, 58%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.67 (1H, s, NH), 7.18 (1H, s, pyrimidine), 1.13-7.12 (3H, m, Ar), 7.04-7.03 (2H, m, Ar), 4.56-4.54 (1H, m, C$\underline{H}$CH$_2$Ph), 4.29-4.24 (1H, m, CH(Cp)), 3.02-2.99 (2H, m, C$\underline{H}_2$Ph), 1.97-1.53 (8H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.6, 151.9, 151.8, 137.8, 135.2, 130.5, 128.2, 127.4, 119.9, 61.4, 59.9, 29.3, 29.0, 24.1, 23.8.

(R)-2-Chloro-7-ethyl-8-isobutyl-7,8-dihydropteridin-6(5H)-one **(22).** Compound **16** was reductively heterocyclized on a 2.4 mmol scale with iron powder and acetic acid according to general procedure D to yield compound **22** as a yellow powder (290 mg, 45%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.88 (1H, s, NH), 7.57 (1H, s, pyrimidine), 4.16 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 3.90 (1H, dd, *J* = 8.0Hz, 13.6Hz, C$\underline{H}_a$CH), 2.83 (1H, dd, *J* = 7.2Hz, 13.6Hz, C$\underline{H}_b$CH), 2.09-2.03 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 1.84-1.77 (2H, m, CHC$\underline{H}_2$CH$_3$), 0.91 (3H, d, *J* = 6.4Hz, CH(C$\underline{H}_3$)$_2$), 0.83 (3H, d, *J* = 7.2Hz, CH(C$\underline{H}_3$)$_2$), 0.77 (3H, t, *J* = 7.2Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR

(DMSO-d_6, 100MHz) δ 164.5, 152.6, 152.3, 138.4, 119.2, 61.9, 51.6, 25.9, 24.8, 20.2, 20.1, 8.9.

(R)-8-(3-Bromobenzyl)-2-chloro-7-ethyl-7,8-dihydropteridin-6(5H)-one **(23).** Compound **17** was reductively heterocyclized on a 4.2 mmol scale with iron powder and acetic acid according to general procedure D to yield compound **23** as a yellow powder (1 g, 62%): ^{1}H NMR (DMSO-d_6, 400MHz) δ (1H, s, NH), 7.62 (2H, s, pyrimidine and Ar), 7.49 (1H, d, J = 7.6Hz, Ar), 7.37 (1H, d, J = 8.0Hz, Ar), 7.31 (1H, t, J = 7.6Hz, Ar), 5.12 (1H, d, J = 15.6Hz, C$\underline{H}_a$Ph), 4.44 (1H, d, J = 14.8Hz, C$\underline{H}_b$Ph), 4.17 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 1.83-1.79 (2H, m, CHC$\underline{H}_2$CH$_3$), 0.73 (3H, t, J = 7.2Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.4, 152.4, 152.0, 139.6, 138.7, 131.2, 130.9, 127.4, 122.2, 119.2, 61.3, 47.4, 24.6, 8.6.

(R)-2-Chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one **(24).** Compound **18** was alkylated with methyl iodide according to general procedure E on a 0.53 mmol scale to provide compound **24** as a light yellow solid (156 mg, quant.): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.86 (1H, s, pyrimidine), 4.34 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.18-4.13 (1H, m, CH(Cp)), 3.24 (1H, s, N-CH$_3$), 1.92-1.76 (6H, m, Cp), 1.72-1.65 (2H, m, CHC$\underline{H}_2$CH$_3$), 1.56-1.53 (2H, m, Cp), 0.73 (3H, t, J = 8.0Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 163.6, 152.6, 151.9, 138.9, 121.7, 61.2, 59.8, 28.8, 28.7, 28.4, 27.1, 24.3, 8.9.

2-Chloro-8-cyclopentyl-5-methyl-7,8-dihydropteridin-6(5H)-one **(25).** Compound **19** was alkylated with methyl iodide according to general procedure E on a scale of 0.98 mmol to provide compound **25** as a white solid (188 mg, 72%): ^{1}H NMR (CDCl$_3$, 400MHz) δ 7.66 (1H, s, pyrimidine), 5.16-5.11 (1H, m, CH(Cp)), 4.12 (2H, s, CH$_2$), 3.32 (3H, s, N-CH$_3$), 1.94-1.56 (8H, m, Cp).

(S)-2-Chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one **(26).** Compound **20** was alkylated with methyl iodide according to general procedure E on a 0.47 mmol scale to provide compound **26** as a light yellow solid (129 mg, 96%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.87 (1H, s, pyrimidine), 4.35-4.33 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.18-4.14 (1H, m, CH(Cp)), 3.24 (3H, s, N-CH$_3$), 1.92-1.53 (10H, m, Cp and CHC$\underline{H}_2$CH$_3$), 0.73 (3H, t, J = 6.8Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 163.6, 152.6, 151.9, 138.9, 121.7, 61.2, 59.8. 28.8, 28.7, 28.4, 27.1, 24.3, 8.9.

(R)-7-Benzyl-2-chloro-8-cyclopentyl-5-methyl-7,8-dihydropteridin-6(5H)-one **(27).** Compound **21** was alkylated with methyl iodide according to general procedure E on a 0.39 mmol scale to provide compound **27** as a light yellow solid (159 mg, quant.): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.36 (1H, s, pyrimidine), 7.09-7.08 (3H, m, Ar), 6.93-6.91 (2H, m, Ar), 4.71-4.69 (1H, m, C$\underline{H}$CH$_2$Ph), 4.35-4.31 (1H, m, CH(Cp)), 3.10-3.00 (5H, m, N-CH$_3$ and C$\underline{H}_2$Ph), 2.03-1.55 (8H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 163.6, 152.5, 137.9, 135.0, 130.3, 128.1, 127.5, 121.4, 60.9, 59.7, 29.3, 29.0, 27.9, 24.1, 23.7.

(R)-2-Chloro-7-ethyl-8-isobutyl-5-methyl-7,8-dihydropteridin-6(5H)-one **(28).** Compound **22** was alkylated with methyl iodide according to general procedure E on a 0.56 mmol scale to provide compound **28** as a light yellow solid (158 mg, quant.): ^{1}H NMR (CDCl$_3$, 400MHz) δ 7.62 (1H, s, pyrimidine), 4.18-4.11 (2H, m, C$\underline{H}$CH$_2$CH$_3$ and C$\underline{H}_a$CH(CH$_3$)$_2$), 3.31 (3H, s, N-CH$_3$), 2.63 (1H, dd, J = 7.2Hz, 14.4Hz, C$\underline{H}_b$CH(CH$_3$)$_2$), 2.07-1.91 (1H, m, CHC$\underline{H}_a$CH$_3$), 1.90-1.85 (1H, m, CH$_2$C$\underline{H}$(CH$_3$)$_2$), 1.80-1.73 (1H, m, CHC$\underline{H}_b$CH$_3$), 0.93 (3H, d, J = 7.2Hz, CH(C$\underline{H}_3$)$_2$), 0.85 (3H, d, J = 6.8Hz, CH(C$\underline{H}_3$)$_2$), 0.81 (3H, t, J = 6.8Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (CDCl$_3$, 100MHz) δ 163.6, 154.1, 152.6, 137.5, 120.5, 62.1, 52.2, 28.2, 26.1, 25.3, 20.1, 19.8, 8.9.

(R)-8-(3-Bromobenzyl)-2-chloro-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one **(29).** Compound **23** was alkylated with methyl iodide according to general procedure E on a 2.62 mmol scale to provide compound **(29)** as a light yellow solid (1 g, quant.): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.92 (1H, s, pyrimidine), 7.63 (1H, s, Ar), 7.48 (1H, d, J = 8.0Hz, Ar), 7.38 (1H, d, J = 7.6Hz, Ar), 7.31 (1H, t, J = 8.0Hz, Ar), 5.14 (1H, d, J = 16.0Hz, C$\underline{H}_a$Ph), 4.43 (1H, d, J = 14.8Hz, C$\underline{H}_b$Ph), 4.29 (1H, t, J = 4.4Hz, C$\underline{H}$CH$_2$CH$_3$), 3.26 (3H, s, N-CH$_3$), 1.82-1.78 (2H, m, CHC$\underline{H}_2$CH$_3$), 0.70 (3H, t, J = 6.8Hz, CHCH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 163.4, 152.8, 152.3, 139.5, 139.2, 131.3, 131.2, 130.9, 127.4, 122.2, 121.2, 79.6, 61.3, 47.5, 28.5, 25.1, 8.7.

(R)-2-Chloro-8-cyclopentyl-5,7-diethyl-7,8-dihydropteridin-6(5H)-one **(30).** Compound **18** was alkylated with ethyl iodide according to general procedure E on a 0.27 mmol scale to provide compound **30** as a light yellow solid (83 mg, quant.): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.94 (1H, s, pyrimidine), 4.33 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.20-4.16 (1H, m, CH(Cp)), 4.02-3.96 (1H, m, N-C$\underline{H}_a$CH$_3$), 3.78-3.73 (1H, m, N-C$\underline{H}_b$CH$_3$), 1.97-1.54 (10H, m, Cp and CHC$\underline{H}_2$CH$_3$), 1.11 (3H, t, J = 7.2Hz, CH$_3$), 0.73 (3H, t, J = 7.2Hz, CH$_3$).

(R)-2-Chloro-8-cyclopentyl-7-ethyl-5-isopropyl-7,8-dihydropteridin-6(5H)-one **(31).** Compound **18** was alkylated with isopropyl iodide according to general procedure E on a 0.31 mmol scale for 16 h to provide compound **31** as a light yellow solid (33 mg, 32%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.06 (1H, s, pyrimidine), 4.70-4.66 (1H, m, CH(Cp)), 4.18-4.12 (2H, m, C$\underline{H}$CH$_2$CH$_3$ and C$\underline{H}$(CH$_3$)$_2$), 1.91-1.51 (10H, m, Cp and CHC$\underline{H}_2$CH$_3$), 1.37 (3H, d, J = 6.8Hz, CH(C$\underline{H}_3$)$_2$), 1.33 (3H, d, J = 6.8Hz, CH(C$\underline{H}_3$)$_2$), 0.69 (3H, t, J = 7.6 Hz, CH$_2$C$\underline{H}_3$).

(R)-2-Chloro-8-cyclopentyl-7-ethyl-5-isobutyl-7,8-dihydropteridin-6(5H)-one **(32).** Compound **18** was alkylated with iodo-2-methylpropane according to general procedure E on a scale of 0.27 mmol for 16 h to provide compound **32** as a light yellow solid (44 mg, 48%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.95 (1H, s, pyrimidine), 4.31-4.28 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.24-4.19 (1H, m, CH(Cp)), 3.82-3.76 (1H, m, N-C$\underline{H}_a$CH), 3.61-3.56 (1H, m, N-C$\underline{H}_b$CH), 2.00-1.54 (11H, m, Cp, C$\underline{H}$(CH$_3$)$_2$ and CHC$\underline{H}_2$CH$_3$), 0.88 (3H, d, J = 7.2Hz, CH(C$\underline{H}_3$)$_2$), 0.84 (3H, d, J = 7.2Hz, CH(C$\underline{H}_3$)$_2$), 0.77 (3H, t, J = 7.2Hz, CH$_2$C$\underline{H}_3$).

(R)-5-Benzyl-2-chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one **(33).** Compound **18** was alkylated with benzyl bromide according to general procedure E on a scale of 0.36 mmol to provide compound **33** as a light yellow solid (50 mg, 36%.): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.76 (1H, s, pyrimidine), 7.37-7.33 (2H, m, Ar), 7.28-7.25 (3H, m, Ar), 5.18 (1H, d, *J* = 15.6Hz, N-C$\underline{H}_a$Ph), 5.03 (1H, d, *J* = 15.6Hz, N-C$\underline{H}_b$Ph), 4.47-4.44 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.24-4.19 (1H, m, CH(Cp)), 1.98-1.72 (8H, m, Cp), 1.57-1.55 (2H, m, CHC$\underline{H}_2$CH$_3$), 0.81 (3H, t, *J* = 6.8Hz, CH$_2$C$\underline{H}_3$).

3-Isobutoxy-4-nitrobenzoic acid **(34e).** 3-Hydroxy-4-nitrobenzoic acid (1 g, 5.46 mmol) was esterified by $SOCl_2$ (2.1 eq) in MeOH (0.1 M), then the product was deprotonated by K_2CO_3 (2.0 eq) in DMF (0.1 M) and alkylated by 1-iodo-2-mythylpropane (0.67 eq), the product was then hydrolyzed by LiOH·H2O (4.0 eq) in a solvent mixture of THF, MeOH and H2O 3:1:1 (0.1 M) to give compound **34e** as a light yellow powder (679 mg, 52%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 13.63 (1H, s, COOH), 7.95 (1H, t, *J* = 7.6Hz, Ar), 7.43 (1H, d, *J* = 8.8Hz, Ar), 7.621 (1H, t, *J* = 8.8Hz, Ar), 4.01 (2H, d, *J* = 7.2Hz, CH$_2$), 2.07-1.99 (1H, m, CH), 0.97 (6H, t, *J* = 6.8Hz, CH(C$\underline{H}_3$)$_2$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 166.2, 151.4, 142.5, 136.1, 125.4, 121.6, 115.6, 75.6, 28.1, 19.1.

3-(Benzyloxy)-4-nitrobenzoic acid **(34f).** 3-Hydroxy-4-nitrobenzoic acid (1 g, 5.46 mmol) was esterified according to general procedure A, then the product was deprotonated by K_2CO_3 (2.0 eq) in DMF (0.1 M) and alkylated by benzyl bromide (0.9 eq). The reaction mixture was partitioned between EtOAc and 1M NaOH, and then the EtOAc extraction was washed with water (x3). The residue was subsequently hydrolyzed by LiOH·H_2O (4.0 eq) in a solvent mixture of THF, MeOH and H_2O 3:1:1 (0.1 M) to give compound **34f** as a light yellow powder (1 g, 70%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 13.7 (1H, s, COOH), 8.00 (1H, d, *J* = 8.8Hz, Ar), 7.88 (1H, s, Ar), 7.66 (1H, d, *J* = 8.0Hz, Ar), 7.48-7.33 (5H, m, Ar), 5.40 (2H, s, CH$_2$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 166.2, 150.9, 142.7, 136.2, 136.1, 128.9, 128.5, 127.8, 125.5, 122.0, 116.3, 71.1.

3-(Cyclopentyloxy)-4-nitrobenzoic acid **(34g).** 3-Hydroxy-4-nitrobenzoic acid (1 g, 5.46 mmol) was esterified by $SOCl_2$ (2.1 eq) in MeOH (0.1 M) according to general procedure A, then the product was alkylated by cyclopentanol (0.8 eq) with PPh_3 (1.3 eq) and diisopropyl azodicarboxylate (1.3 eq). After 16h at RT, the product was isolated by flash column chromatography (eluent: Hex/EtOAc, 4:1), then hydrolyzed by LiOH·H_2O (4.0 eq) in a solvent mixture of THF, MeOH and H_2O 3:1:1 (0.1 M) to give compound **34g** as a light yellow powder (925 mg, 64%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 13.65 (1H, s, COOH), 7.92 (1H, d, *J* = 7.6Hz, Ar), 7.75 (1H, s, Ar). 7.61 (1H, d, *J* = 8.4Hz, Ar), 5.20-5.10 (1H, m, CH), 1.93-1.58 (8H, m, Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 166.2, 150.2, 143.4, 135.9, 125.4, 121.5, 116.8, 81.8, 32.5, 23.8.

3-Methoxy-N-(1-methylpiperidin-4-yl)-4-nitrobenzamide **(35a).** 3-Methoxy-4-nitrobenzoic acid (**34a**) was coupled to 4-amino-1-methylpiperidine in CH_2Cl_2 with HBTU (1.6 eq) and triethylamine (2.0 eq) on a scale of 2.5 mmol according to general procedure F to provide compound **35a** as a yellow powder (660 mg, 90%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.61 (1H, d, *J* = 7.2Hz, Ar), 7.97 (1H, d, *J* = 8.4Hz, NH), 7.69 (1H, s, Ar), 7.56 (1H, d, *J* = 6.8Hz, Ar), 3.99 (3H, s, O-CH$_3$), 3.94-3.91 (1H, m, NHC$\underline{H}$), 3.16 (2H, d, *J* = 12.0Hz, piperidine), 2.69 (3H, s, N-CH$_3$), 2.64-2.61 (4H, m, piperidine), 1.96-1.92 (2H, m, piperidine), 1.76-1.70 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.6, 152.0, 141.2, 139.9, 125.3, 119.8, 113.5, 57.3, 53.6, 45.8, 44.3, 38.7, 30.1.

N-(1-Methylpiperidin-4-yl)-4-nitrobenzamide **(35b).** 4-Nitrobenzoic acid (**34b**) was coupled to 4-amino-1-methylpiperidine in CH_2Cl_2 with HBTU (1.6 eq) and triethylamine (2.0 eq) on a scale of 5.9 mmol according to general procedure F to provide compound **35b** as a yellow powder (1.4 g, 92%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.56 (1H, d, *J* = 7.6Hz, NH), 8.27 (2H, d, *J* = 8.8Hz, Ar), 8.03 (2H, d, *J* = 8.8Hz, Ar), 3.72-3.68 (1H, m, NHC$\underline{H}$), 2.73 (2H, d, *J* = 11.6Hz, piperidine), 2.13 (3H, s, N-CH$_3$), 1.90 (2H, t, *J* = 12.0Hz, piperidine), 1.74 (2H, t, *J* = 11.2Hz, piperidine), 1.59-1.49 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.4, 149.3, 140.8, 129.2, 123.8, 54.8, 47.3, 46.4, 31.7.

2-Methoxy-N-(1-methylpiperidin-4-yl)-4-nitrobenzamide **(35c).** 2-Methoxy-4-nitrobenzoic acid (**34c**) was coupled to 4-amino-1-methylpiperidine in CH_2Cl_2 with HBTU (1.6 eq) and triethylamine (2.0 eq) on a scale of 1.5 mmol according to general procedure F to provide compound **35c** as a yellow powder (307 mg, 70%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.25 (1H, d, *J* = 8.0Hz, NH), 7.86-7.84 (2H, m, Ar), 7.69 (1H, d, *J* = 8.8Hz, Ar), 3.95 (3H, s, O-CH$_3$), 3.78-3.76 (1H, m, NHC$\underline{H}$), 2.82 (2H, d, *J* = 12.0Hz, piperidine), 2.27 (3H, s, N-CH$_3$), 2.21 (2H, t, *J* = 10.8Hz, piperidine), 1.83 (2H, d, *J* = 10.8Hz, piperidine), 1.61-1.51 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.1, 157.3, 149.5, 131.9, 130.7, 115.8, 107.2, 57.0, 54.0, 46.0, 45.7, 38.7, 31.1.

3-Fluoro-N-(1-methylpiperidin-4-yl)-4-nitrobenzamide **(35d).** 3-Fluoro-4-nitrobenzoic acid (**34d**) was coupled to 4-amino-1-methylpiperidine in CH_2Cl_2 with HBTU (1.6 eq) and triethylamine (2.0 eq) on a scale of 2.5 mmol according to general procedure F to provide compound **35d** as a light yellow powder (1.4 g, 94%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.58 (1H, d, *J* = 8.0Hz, NH), 8.22 (1H, t, *J* = 8.0Hz, Ar), 7.93 (1H, d, *J* = 12.0Hz, Ar), 7.83 (1H, d, *J* = 8.4Hz, Ar), 3.71-3.66 (1H, m, NHC$\underline{H}$), 2.73 (2H, d, *J* = 11.6Hz, piperidine), 2.12 (3H, s, N-CH$_3$), 1.90 (2H, t, *J* = 10.8Hz, piperidine), 1.74 (2H, d, *J* = 12.0Hz, piperidine), 1.57-1.49 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 163.1, 156.0, 153.4, 141.9, 126.8, 124.4, 117.8, 117.5, 54.8, 47.5, 46.4, 31.7.

3-Isobutoxy-N-(1-methylpiperidin-4-yl)-4-nitrobenzamide **(35e).** Compound **34e** was coupled to 4-amino-1-

methylpiperidine in CH_2Cl_2 with HBTU (1.6 eq) and triethylamine (2.0 eq) on a scale of 1.3 mmol according to general procedure F to provide compound **35e** as a yellow powder (335 mg, 76%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.48 (1H, d, *J* = 8.0Hz, NH), 7.93 (1H, d, *J* = 8.4Hz, Ar), 7.65 (1H, s, Ar), 7.53 (1H, d, *J* = 8.4Hz, Ar), 3.99 (2H, d, *J* = 6.4Hz, OC<u>H</u>$_2$), 3.75-3.69 (1H, m, NHC<u>H</u>), 2.77 (2H, d, *J* = 11.6Hz, piperidine), 2.17 (3H, s, N-CH_3), 2.07-1.53 (7H, m, piperidine and C<u>H</u>$(CH_3)_2$), 0.99 (6H, d, *J* = 6.0Hz, CH(C<u>H</u>$_3$)$_2$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.3, 151.5, 141.2, 140.1, 125.2, 119.7, 114.1, 75.6, 54.9, 47.4, 46.4, 38.7, 31.8, 28.1, 19.2.

3-(Benzyloxy)-N-(1-methylpiperidin-4-yl)-4-nitrobenzamide **(35f).** Compound **34f** was coupled to 4-amino-1-methylpiperidine in CH_2Cl_2 with HBTU (1.6 eq) and triethylamine (2.0 eq) on a scale of 3.6 mmol according to general procedure F to provide compound **35f** as a yellow powder (1.2 g, 91%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.51 (1H, d, *J* = 8.0Hz, NH), 7.97 (1H, d, *J* = 8.8Hz, Ar), 7.82 (1H, s, Ar), 7.57 (1H, d, *J* = 7.6Hz, Ar), 7.48-7.33 (5H, m, Ar), 5.37 (2H, s, C<u>H</u>$_2$Ph), 3.76-3.72 (1H, m, NHC<u>H</u>), 2.79 (2H, d, *J* = 11.6Hz, piperidine), 2.18 (3H, s, N-CH_3), 1.99 (2H, t, *J* = 11.6Hz, piperidine), 1.78 (2H, d, *J* = 10.8Hz, piperidine), 1.65-1.55 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.2, 151.1, 141.4, 140.1, 136.2, 128.9, 128.6, 127.9, 125.3, 120.1, 114.7, 71.1, 54.8, 47.3, 46.3, 31.7.

3-(Cyclopentyloxy)-N-(1-methylpiperidin-4-yl)-4-nitrobenzamide **(35g).** Compound **34g** was coupled to 4-amino-1-methylpiperidine in CH_2Cl_2 with HBTU (1.6 eq) and triethylamine (2.0 eq) on a scale of 3.3 mmol according to general procedure F to provide compound **35g** as a yellow powder (1.1 g, 96%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.50 (1H, d, *J* = 7.6Hz, NH), 7.90 (1H, d, *J* = 8.4Hz, Ar), 7.67 (1H, s, Ar), 7.52 (1H, d, *J* = 7.6Hz, Ar), 5.20-5.12 (1H, m, OCH), 3.77-3.73 (1H, m, NHC<u>H</u>), 2.82 (2H, d, *J* = 12.0Hz, piperidine), 2.19 (3H, s, N-CH_3), 2.04-1.56 (16H, m, piperidine and Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.3, 150.3, 142.2, 139.8, 125.1, 119.6, 115.4, 81.7, 54.7, 47.2, 46.1, 38.7, 32.6, 31.6, 23.8.

4-Amino-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(36a).** Compound **35a** was reduced by stannous chloride dihydrate on a scale of 4.3 mmol according to general procedure G to give compound **36a** as a light yellow powder (1 g, 89%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.78 (1H, d, *J* = 8.0Hz, NH), 7.30-7.27 (2H, m, Ar), 6.58 (1H, d, *J* = 8.8Hz, Ar), 5.21 (2H, s, NH_2), 3.79 (3H, s, O-CH_3), 3.71-3.68 (1H, m, NHC<u>H</u>), 2.75 (2H, d, *J* = 10.8Hz, piperidine), 2.15 (3H, s, N-CH_3), 1.91 (2H, t, *J* = 10.8Hz, piperidine), 1.73-1.70 (2H, m, piperidine), 1.60-1.50 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.9, 145.6, 141.2, 122.3, 121.5, 112.4, 110.0, 55.8, 55.1, 46.7, 46.5, 32.2.

4-Amino-N-(1-methylpiperidin-4-yl)benzamide **(36b).** Compound **35b** was reduced by stannous chloride dihydrate on a scale of 3.4 mmol according to general procedure G to give compound **36b** as a light yellow powder (673 mg, 85%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.71 (1H, d, *J* = 7.6Hz, NH), 7.56 (2H, d, *J* = 8.4Hz, Ar), 6.51 (2H, d, *J* = 7.6Hz, Ar), 5.56 (2H, s, NH_2), 3.71-3.63 (1H, m, NHC<u>H</u>), 2.74 (2H, d, *J* = 11.6Hz, piperidine), 2.15 (3H, s, N-CH_3), 1.90 (2H, t, *J* = 10.8Hz, piperidine), 1.70 (2H, d, *J* = 10.4Hz, piperidine), 1.58-1.48 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 166.0, 151.9, 129.2, 121.9, 112.8, 55.1, 46.6, 46.5, 32.1.

4-Amino-2-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(36c).** Compound **35c** was reduced by stannous chloride dihydrate on a scale of 1.0 mmol according to general procedure G to give compound **36c** as a light yellow powder (231 mg, 88%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.64 (1H, d, *J* = 7.2Hz, NH), 7.56 (1H, d, *J* = 8.8Hz, Ar), 6.19 (1H, s, Ar), 6.15 (1H, d, *J* = 8.4Hz, Ar), 5.68 (2H, s, NH_2), 3.79 (3H, s, O-CH_3), 3.75-3.69 (1H, m, NHC<u>H</u>), 2.60-2.56 (2H, m, piperidine), 2.12 (3H, s, N-CH_3), 2.02 (2H, t, *J* = 10.0Hz, piperidine), 1.75 (2H, d, *J* = 10.0Hz, piperidine), 1.49-1.41 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.3, 159.2, 153.5, 132.8, 109.3, 106.5, 96.4, 55.8, 54.2, 46.4, 45.4, 31.9.

4-Amino-3-fluoro-N-(1-methylpiperidin-4-yl)benzamide **(36d).** Compound **35d** was reduced by stannous chloride dihydrate on a scale of 4.5 mmol according to general procedure G to give compound **36d** as a light yellow powder (904 mg, 80%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.85 (1H, d, *J* = 8.0Hz, NH), 7.52 (1H, d, *J* = 12.4Hz, Ar), 7.45 (1H, d, *J* = 8.8Hz, Ar), 6.72 (1H, t, *J* = 8.8Hz, Ar), 5.66 (2H, s, NH_2), 3.69-3.62 (1H, m, NHC<u>H</u>), 2.75 (2H, d, *J* = 10.8Hz, piperidine), 2.15 (3H, s, N-CH_3), 1.91 (2H, t, *J* = 11.2Hz, piperidine), 1.70 (2H, d, *J* = 10.4Hz, piperidine), 1.58-1.49 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.9, 151.0, 148.7, 139.9, 139.7, 124.8, 122.2, 115.0, 114.5, 114.3, 55.0, 46.8, 46.4, 32.0.

4-Amino-3-isobutoxy-N-(1-methylpiperidin-4-yl)benzamide **(36e).** Compound **35e** was reduced by stannous chloride dihydrate on a scale of 0.66 mmol according to general procedure G to give compound **36e** as a light yellow powder (191 mg, 95%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.78 (1H, d, *J* = 8.0Hz, NH), 7.27 (2H, m, Ar), 6.60 (1H, d, *J* = 7.6Hz, Ar), 5.16 (2H, s, NH_2), 3.74 (2H, d, *J* = 6.0Hz, OCH_2), 3.71-3.66 (1H, m, NHC<u>H</u>), 2.76 (2H, d, *J* = 12.0Hz, piperidine), 2.15 (3H, s, N-CH_3), 2.09-2.03 (1H, m, C<u>H</u>$(CH_3)_2$), 1.91 (2H, t, *J* = 12.0Hz, piperidine), 1.71 (2H, d, *J* = 10.8Hz, piperidine), 1.59-1.51 (2H, m, piperidine), 1.01 (6H, d, *J* = 6.4Hz, CH(C<u>H</u>$_3$)$_2$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.9, 145.0, 141.3, 121.4, 112.5, 110.8, 74.5, 55.2, 46.7, 46.5, 39.2, 38.7, 32.1, 28.3, 19.7.

4-Amino-3-(benzyloxy)-N-(1-methylpiperidin-4-yl)benzamide **(36f).** Compound **35f** was reduced by stannous chloride dihydrate on a scale of 4.5 mmol according to general procedure G to give compound **36f** as a light yellow powder (1.4 g, 93%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.79 (1H, d, *J* = 7.6Hz, NH), 7.51 (2H, d, *J* = 6.8Hz, Ar), 7.42-7.7.37 (3H, m, Ar), 7.34-7.30 (2H, m, Ar), 6.63 (1H, d, *J* = 8.4Hz, Ar), 5.25 (2H, s, NH_2), 5.13 (2H, s, C<u>H</u>$_2$Ph), 3.71-3.67 (1H, m, NHC<u>H</u>), 2.77 (2H, d, *J* = 11.6Hz, piperidine),

2.16 (3H, s, N-CH_3), 1.93 (2H, t, *J* = 11.2Hz, piperidine), 1.72 (2H, d, *J* = 10.8Hz, piperidine), 1.59-1.50 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.9, 144.7, 141.5, 137.7, 128.8, 128.1, 127.8, 122.4, 121.8, 112.8, 111.7, 69.9, 55.1, 46.7, 46.4, 38.7, 32.1.

4-Amino-3-(cyclopentyloxy)-N-(1-methylpiperidin-4-yl)benzamide **(36g).** Compound **35g** was reduced by stannous chloride dihydrate on a scale of 3.9 mmol according to general procedure G to give compound **36g** as a light yellow powder (1.1 g, 90%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.86 (1H, d, *J* = 8.0Hz, NH), 7.29-7.27 (2H, m, Ar), 6.58 (1H, d, *J* = 8.4Hz, Ar), 5.13 (2H, s, NH_2), 4.81-4.60 (1H, m, OC<u>H</u>), 3.79-3.77 (1H, m, NHC<u>H</u>), 2.96 (2H, d, *J* = 10.8Hz, piperidine), 2.34 (3H, s, N-CH_3), 1.87-1.57 (14H, m, piperidine and Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 166.1, 143.8, 142.2, 122.2, 121.4, 112.6, 79.6, 54.3, 45.9, 45.2, 38.7, 32.8, 31.1, 24.0.

4-Hydroxy-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(38).** 4-Hydroxy-3-methoxybenzoic acid **(37)** (456 mg, 3 mmol, 1.0 eq) was coupled to 4-amino-1-methylpiperidine (343 mg, 3 mmol, 1.0 eq) with EDCI·HCl (633 mg, 3.3 mmol, 1.1 eq), HOBt·H_2O (4.5 mmol, 1.5 eq), and triethylamine (1.34 mL, 9.6 mmol, 3.2 eq) in acetonitrile (30 mL). After stirring overnight at RT, the reaction mixture was concentrated to dryness. The residue was purified by prepTLC (eluent: CH_2Cl_2/MeOH/H_2O, 79:9:1) to give compound **38** as a white powder (80 mg, 10%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.60 (1H, s, OH), 7.98 (1H, d, *J* = 7.6Hz, NH), 7.40 (1H, s, Ar), 7.35 (1H, d, *J* = 8.0Hz, Ar), 6.78 (1H, d, *J* = 8.4Hz, Ar), 3.80 (3H, s, O-CH_3), 3.74-3.69 (1H, m, NHC<u>H</u>), 2.80 (2H, d, *J* = 12.0Hz, piperidine), 2.19 (3H, s, N-CH_3), 1.98 (2H, t, *J* = 11.2Hz, piperidine), 1.74 (2H, d, *J* = 12.0Hz, piperidine), 1.61-1.55 (2H, m, piperidine); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.4, 149.5, 147.1, 125.7, 120.9, 114.8, 111.5, 55.8, 54.6, 46.4, 45.9, 31.5.

(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(1).** Compound **24** (0.24 mmol) was coupled to compound **36a** (0.38 mmol) according to general procedure H to yield compound **1** as a white solid (50 mg, 40%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.42 (1H, d, *J* = 8.8Hz, CONH), 8.10 (1H, d, *J* = 8.4Hz, Ar), 7.86 (1H, s, pyrimidine), 7.61 (1H, s, Ar), 7.50-7.48 (2H, m, Ar and NH), 4.39-4.34 (1H, m, CH(Cp)), 4.26-4.23 (1H, m, C<u>H</u>CH_2CH_3), 3.94 (3H, s, O-CH_3), 3.77-3.73 (1H, m, NHC<u>H</u>), 3.26 (3H, s, N-CH_3), 2.80 (2H, d, *J* = 11.2Hz, piperidine), 2.18 (3H, s, N-CH_3), 2.02- 1.54 (16H, m, Cp, piperidine and CHC<u>H</u>$_2$$CH_3$), 0.77 (3H, t, *J* = 7.2Hz, CH_2C<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.6, 163.4, 154.8, 151.9, 147.0, 138.8, 132.5, 127.2, 120.5, 116.5, 116.4, 109.6, 60.2, 58.7, 56.5, 55.1, 46.9, 46.4, 32.0, 29.2, 28.9, 28.2, 26.9, 23.7, 23.4, 9.3; MS (APCI+) m/z Calcd (M^+): 521.3, Found: 522.3 ($M+H^+$); t_R = 1.50 min (98.6%).

(R)-4-((8-Cyclopentyl-7-ethyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39a).** Compound **18** (0.18 mmol) was coupled to compound **36a** (0.18 mmol) according to general procedure H to yield compound **39a** as a white solid (30 mg, 32%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.53 (1H, s, N<u>H</u>CO), 8.37 (1H, d, *J* = 8.8Hz, N<u>H</u>CH), 8.07 (1H, d, *J* = 7.2Hz, Ar), 7.56 (1H, s, pyrimidine), 7.53 (1H, s, Ar), 7.47 (2H, m, Ar and NH), 4.32 (1H, m, CH(Cp)), 4.14-4.11 (1H, m, C<u>H</u>CH_2CH_3), 3.93 (3H, s, O-CH_3), 3.74-3.72 (1H, m, NHC<u>H</u>), 2.79 (2H, d, *J* = 10.0Hz, piperidine), 2.17 (3H, s, N-CH_3), 2.02-1.54 (16H, m, piperidine, Cp and CHC<u>H</u>$_2$$CH_3$), 0.80 (3H, t, *J* = 7.6Hz, $CHCH_2$C<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.1, 164.0, 154.4, 150.9, 146.5, 137.9, 132.2, 126.5, 120.1, 115.7, 113.8, 109.2, 60.1, 58.5, 56.0, 46.5, 45.9, 31.6, 28.7, 28.3, 26.4, 23.2, 23.0, 8.7; MS (APCI+) m/z Calcd (M^+): 507.3, Found: 508.3 ($M+H^+$); t_R = 1.46 min (100%).

(R)-4-((8-Cyclopentyl-5,7-diethyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39b).** Compound **30** (0.13 mmol) was coupled to compound **36a** (0.13 mmol) according to general procedure H to yield compound **39b** as a white solid (20 mg, 28%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.37 (1H, d, *J* = 9.2Hz, CONH), 8.04 (1H, d, *J* = 7.6Hz, Ar), 7.86 (1H, s, pyrimidine), 7.56 (1H, s, Ar), 7.50-7.40 (2H, m, Ar and NH), 4.33-4.30 (1H, m, CH(Cp)), 4.19-4.16 (1H, m, C<u>H</u>CH_2CH_3), 3.99- 3.90 (4H, m, N-C<u>H</u>$_a$$CH_3$ and O-CH_3), 3.80-3.60 (2H, m, N-C<u>H</u>$_b$$CH_3$ and NHC<u>H</u>), 2.74 (2H, d, *J* = 10.4Hz, piperidine), 2.13 (3H, s, N-CH_3), 1.98-1.40 (16H, m, Cp, piperidine and CHC<u>H</u>$_2$$CH_3$), 1.09 (3H, t, *J* = 6.4Hz, N-CH_2C<u>H</u>$_3$), 0.73 (3H, t, *J* = 7.6Hz, $CHCH_2$C<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.1, 162.5, 154.2, 151.8, 146.6, 137.9, 132.1, 126.7, 120.1, 115.9, 114.5, 109.2, 59.5, 58.2, 56.0, 54.6, 46.5, 45.9, 35.8, 35.6, 31.6, 28.8, 28.5, 26.3, 23.4, 23.3, 22.9, 12.1, 8.8; MS (APCI+) m/z Calcd (M^+): 535.3, Found: 536.3 ($M+H^+$); t_R = 1.46 min (91.1%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-isopropyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39c).** Compound **31** (0.1 mmol) was coupled to compound **36a** (0.1 mmol) according to general procedure H to yield compound **39c** as a white solid (22 mg, 40%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.37 (1H, d, *J* = 9.2Hz, CONH), 8.06 (1H, d, *J* = 6.8Hz, Ar), 8.01 (1H, s, pyrimidine), 7.57 (1H, s, Ar), 7.45-7.43 (2H, m, Ar and NH), 4.71-4.67 (1H, m, C<u>H</u>$(CH_3)_2$), 4.35-4.31 (1H, m, CH(Cp)), 4.06-4.03 (1H, m, C<u>H</u>CH_2CH_3), 3.90 (3H, s, O-CH_3), 3.80-3.60 (1H, m, NHC<u>H</u>), 2.90-2.70 (2H, m, piperidine), 2.19 (3H, s, N-CH_3), 1.96-1.49 (16H, m, Cp, piperidine and CHC<u>H</u>$_2$$CH_3$), 1.39 (3H, d, *J* = 6.8Hz, CH(C<u>H</u>$_3$)$_2$), 1.34 (3H, d, *J* = 6.8Hz, CH(C<u>H</u>$_3$)$_2$), 0.73 (3H, t, *J* = 6.8Hz, $CHCH_2$C<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.2, 163.8, 154.0, 152.6, 146.6, 139.3, 132.1, 126.7, 120.1, 115.9, 114.8, 109.2, 59.9, 57.9, 56.1, 54.3, 46.1, 45.4, 31.2, 29.0, 28.8, 25.4, 23.3, 22.9, 20.1, 18.5, 9.1; MS (APCI+) m/z Calcd (M^+): 549.3, Found: 550.4 ($M+H^+$); t_R = 1.64 min (100%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-isobutyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39d).** Compound **32** (0.13 mmol) was coupled to compound **36a** (0.13 mmol) ac-

cording to general procedure H to yield compound **39d** as a white solid (30 mg, 40%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.37 (1H, d, *J* = 9.2Hz, CONH), 8.06 (1H, d, *J* = 6.8Hz, Ar), 8.01 (1H, s, pyrimidine), 7.57 (1H, s, Ar), 7.45-7.43 (2H, m, Ar and NH), 4.40-4.36 (1H, m, CH(Cp)), 4.15-4.12 (1H, m, C<u>H</u>CH$_2$CH$_3$), 3.90 (3H, s, O-CH$_3$), 3.77-3.69 (2H, m, NHC<u>H</u> and N-C<u>H$_a$</u>CH), 3.60-3.55 (1H, m, N-C<u>H$_b$</u>CH), 2.77-2.74 (2H,d, *J* = 10.8Hz, piperidine), 2.14 (3H, s, N-CH$_3$), 1.99-1.51 (17H, m, Cp, piperidine, C<u>H</u>(CH$_3$)$_2$ and CHC<u>H$_2$</u>CH$_3$), 0.86 (3H, d, *J* = 6.4Hz, CH(C<u>H$_3$</u>)$_2$), 0.79 (3H, d, *J* = 6.4Hz, CH(C<u>H$_3$</u>)$_2$), 0.77 (3H, t, *J* = 8.0Hz, CH$_2$C<u>H$_3$</u>); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.1, 163.1, 154.2, 151.7, 146.1, 138.9, 136.0, 132.1, 126.7, 120.1, 115.9, 115.4, 109.2, 59.5, 58.0, 56.0, 54.6, 47.3, 46.5, 45.9, 31.6, 28.8, 28.7, 26.0, 25.9, 23.2, 22.8, 19.8, 19.6, 9.1; MS (APCI+) m/z Calcd (M^+): 563.4, Found: 564.4 ($M+H^+$); t_R = 1.62 min (100%).

(R)-4-((5-Benzyl-8-cyclopentyl-7-ethyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39e).** Compound **33** (0.13 mmol) was coupled to compound **36a** (0.13 mmol) according to general procedure H to yield compound **39e** as a white solid (32 mg, 41%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.29 (1H, d, *J* = 8.4Hz, CONH), 8.03 (1H, d, *J* = 8.0Hz, Ar), 7.68 (1H, s, pyrimidine), 7.53 (1H, s, Ar), 7.42-7.40 (2H, m, Ar and NH), 7.33-7.30 (2H, m, Ar), 7.23 (3H, d, *J* = 6.8Hz, Ar), 5.18 (1H, d, *J* = 15.6Hz, N-C<u>H$_a$</u>Ph), 5.03 (1H, d, *J* = 15.6Hz, N-C<u>H$_b$</u>Ph), 4.39-4.35 (1H, m, C<u>H</u>(Cp)), 4.31-4.28 (1H, m, C<u>H</u>CH$_2$CH$_3$), 3.87 (3H, s, O-CH$_3$), 3.69-3.66 (1H, m, NHC<u>H</u>), 2.74 (2H, d, *J* = 11.2Hz, piperidine), 2.12 (1H, s, N-CH$_3$), 1.99-1.49 (16H, m, Cp, piperidine and CHC<u>H$_2$</u>CH$_3$), 0.81 (3H, t, *J* = 7.2Hz, CHCH$_2$C<u>H$_3$</u>); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.1, 163.4, 154.3, 151.8, 146.7, 138.9, 136.4, 131.9, 128.7, 127.3, 126.9, 126.7, 120.1, 116.1, 114.8, 109.2, 59.7, 58.3, 56.0, 54.6, 46.5, 45.9, 43.7, 31.6, 28.9, 28.6, 26.3, 23.3, 22.9, 9.1; MS (APCI+) m/z Calcd (M^+): 597.3, Found: 598.3 ($M+H^+$); t_R = 1.62 min (96.1%).

4-((8-Cyclopentyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39f).** Compound **25** (0.22 mmol) was coupled to compound **36a** (0.22 mmol) according to general procedure H to yield compound **39f** as a white solid (35 mg, 32%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.45 (1H, d, *J* = 8.4Hz, NH), 8.06 (1H, d, J = 7.6Hz, Ar), 7.79 (1H, s, pyrimidine), 7.58 (1H, s, Ar), 7.49-7.47 (2H, m, NH and Ar), 5.04-5.00 (1H, m, C<u>H</u>(Cp)), 4.07 (2H, s, COC<u>H$_2$</u>), 3.94 (3H, s, O-CH$_3$), 3.75-3.72 (1H, m, NHC<u>H</u>), 3.21 (3H, s, N-CH$_3$), 2.78 (2H, d, *J* = 9.6Hz, piperidine), 2.17 (3H, s, N-CH$_3$), 1.97-1.55 (14H, m, piperidine and Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.6, 161.8, 154.8, 151.7, 146.9, 138.3, 132.6, 126.9, 120.6, 116.4, 115.9, 109.6, 56.4, 55.1, 54.9, 46.9, 46.4, 45.1, 32.1, 27.8, 27.0, 24.3; MS (APCI+) m/z Calcd (M^+): 493.3, Found: 494.3 ($M+H^+$); t_R = 1.47 min (100%).

(S)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39g).** Compound **26** (0.22 mmol) was coupled to compound **36a** (0.22mmol) according to general procedure H to yield compound **39g** as a white solid (38 mg, 33%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.41 (1H, d, *J* = 8.8Hz, NH), 8.08 (1H, d, *J* = 7.6Hz, Ar), 7.84 (1H, s, pyrimidine), 7.59 (1H, s, NH), 7.48-7.47 (2H, m, Ar), 4.37-4.35 (1H, m, C<u>H</u>(Cp)), 4.23 (1H, m, C<u>H</u>CH$_2$CH$_3$), 3.94 (3H, s, O-CH$_3$), 3.74-3.72 (1H, m, NHC<u>H</u>), 3.24 (3H, s, N-CH$_3$), 2.79 (2H, d, *J* = 10.8Hz, piperidine), 2.18 (3H, s, N-CH$_3$), 2.00-1.54 (16H, m, piperidine, Cp and CHC<u>H$_2$</u>CH$_3$), 0.76 (3H, t, *J* = 6.8Hz, CHCH$_2$C<u>H$_3$</u>); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.6, 163.3, 154.7, 151.9, 147.1, 138.8, 132.6, 127.2, 120.5, 116.5, 116.4, 109.7, 60.2, 58.7, 56.5, 46.9, 46.4, 32.0, 29.2, 28.9, 28.2, 26.9, 23.7, 23.4, 9.3; MS (APCI+) m/z Calcd (M^+): 521.3, Found: 522.3 ($M+H^+$); t_R = 1.55 min (100%).

(R)-4-((7-Benzyl-8-cyclopentyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39h).** Compound **27** (0.19 mmol) was coupled to compound **36a** (0.19 mmol) according to general procedure H to yield compound **39h** as a white solid (26 mg, 23%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.38 (1H, d, *J* = 8.8Hz, NH), 8.09 (1H, d, *J* = 7.2Hz, Ar), 7.52-7.47 (3H, m, Ar and NH), 7.42 (1H, s, pyrimidine), 7.12-7.05 (3H, m, Ar), 7.00-6.95 (2H, m, Ar), 4.60-4.52 (1H, m, C<u>H</u>CH$_2$Ph), 4.46-4.42 (1H, m, C<u>H</u>(Cp)), 3.94 (3H, s, O-CH$_3$), 3.80-3.76 (1H, m, NHC<u>H</u>), 3.04 (3H, s, N-CH$_3$), 3.96-3.92 (2H, m, CHC<u>H$_2$</u>Ph), 2.82 (2H, d, *J* = 10.0Hz, piperidine), 2.21 (3H, s, N-CH$_3$), 2.03-1.59 (14H, m, piperidine and Cp); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.6, 163.1, 154.4, 152.1, 146.9, 137.9, 135.7, 132.7, 130.2, 128.1, 127.3, 126.9, 120.5, 116.4, 116.0, 109.6, 60.4, 58.9, 56.5, 55.0, 46.9, 46.3, 31.9, 29.5, 29.2, 27.9, 23.7, 23.1; MS (APCI+) m/z Calcd (M^+): 583.3, Found: 584.3 ($M+H^+$); t_R = 1.55 min (96.7%).

(R)-4-((7-Ethyl-8-isobutyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39i).** Compound **28** (0.22 mmol) was coupled to compound **36a** (0.22 mmol) according to general procedure H to yield compound **39i** as a white solid (30 mg, 27%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.40 (1H, d, *J* = 9.2Hz, NH), 8.06 (1H, d, *J* = 8.4Hz, Ar), 7.79 (1H, s, pyrimidine), 7.59 (1H, s, Ar), 7.46-7.44 (2H, m, NH and Ar), 4.15 (1H, t, *J* = 5.2Hz, C<u>H</u>CH$_2$CH$_3$), 4.00 (1H, dd, *J* = 6.8Hz, 14.0Hz, C<u>H$_a$</u>CH(CH$_3$)$_2$), 3.91 (3H, s, O-CH$_3$), 3.72 (1H, m, NHC<u>H</u>), 3.24 (3H, s, N-CH$_3$), 2.81-2.75 (3H, m, piperidine and C<u>H$_b$</u>CH(CH$_3$)$_2$)), 2.15 (3H, s, N-CH$_3$), 2.08-2.04 (1H, m, CHC<u>H$_a$</u>CH$_3$), 1.93 (2H, m, piperidine), 1.74-1.70 (4H, m, piperidine and CHC<u>H$_b$</u>CH$_3$), 1.58-1.52 (2H, m, piperidine), 0.89 (6H, t, *J* = 8.0Hz, CH(C<u>H$_3$</u>)$_2$), 0.72 (3H, t, *J* = 7.2Hz, CHCH$_2$C<u>H$_3$</u>); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.5, 163.2, 154.9, 151.9, 146.9, 138.8, 132.6, 127.1, 120.5, 116.2, 115.8, 109.6, 62.5, 56.5, 55.0, 52.2, 46.9, 46.6, 32.0, 28.2, 26.5, 25.1, 20.4, 20.3, 9.3; MS (APCI+) m/z Calcd (M^+): 509.3, Found: 510.3 ($M+H^+$); t_R = 1.49 min (98.3%).

(R)-4-((8-(3-Bromobenzyl)-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39j).** Compound **29** (0.15 mmol) was coupled to compound **36a** (0.15 mmol) according to general procedure H to yield compound **39j** as

a white solid (49 mg, 52%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.26 (1H, d, *J* = 8.8Hz, Ar), 8.04 (1H, d, *J* = 7.6Hz, Ar), 7.88 (1H, s, pyrimidine), 7.64 (2H, d, *J* = 14.8Hz, Ar), 7.49-7.37 (4H, m, Ar), 7.31 (1H, t, *J* = 8.0Hz, Ar), 5.27 (1H, d, *J* = 15.6Hz, C$\underline{H}_a$Ph), 4.43 (1H, d, *J* = 16.0Hz, C$\underline{H}_b$Ph), 4.19 (1H, t, *J* = 4.0Hz, C$\underline{H}$CH$_2$CH$_3$), 3.92 (3H, s, O-CH$_3$), 3.74-3.72 (1H, m, NHC$\underline{H}$), 3.27 (3H, s, N-CH$_3$), 2.80 (2H, d, *J* = 10.0 Hz, piperidine), 2.19 (3H, s, N-CH$_3$), 2.10-1.90 (2H, m, piperidine), 1.80-1.70 (4H, m, piperidine and CHC$\underline{H}_2$CH$_3$), 1.62-1.50 (2H, m, piperidine), 0.73 (3H, t, *J* = 7.2Hz, CHCH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.7, 163.1, 154.9, 151.7, 147.1, 138.9, 132.4, 131.2, 130.9, 130.7, 127.0, 122.3, 120.7, 116.6, 115.8, 112.8, 109.7, 79.6, 61.4, 56.5, 54.3, 47.3, 45.2, 31.0, 28.3, 24.9, 8.9; MS (APCI+) m/z Calcd (M(^{79}Br)$^+$): 621.2, Found: 622.2 (M(^{79}Br)+H$^+$); t_R = 1.55 min (100%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-N-(1-methylpiperidin-4-yl)benzamide **(39k).** Compound **24** (0.20 mmol) was coupled to compound **36b** (0.20 mmol) according to general procedure H to yield compound **39k** as a white solid (46 mg, 46%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.27 (1H, s, pyrimidine), 7.94 (1H, d, *J* = 8.0sHz, NH), 7.81 (1H, s, NH), 7.75 (2H, d, *J* = 8.8Hz, Ar), 7.70 (2H, d, *J* = 8.8Hz, Ar), 4.42-4.37 (1H, m, CH(Cp)), 4.18-4.13 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 3.70-3.67 (1H, m, NHC$\underline{H}$), 3.21 (3H, s, N-CH$_3$), 2.73 (2H, d, *J* = 11.2Hz, piperidine), 2.13 (3H, s, N-CH$_3$), 1.98-1.48 (16H, m, piperidine, Cp and CHC$\underline{H}_2$CH$_3$), 0.74 (3H, t, *J* = 7.6Hz, CHCH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.3, 162.9, 154.9, 151.6, 143.9, 138.5, 127.9, 126.1, 116.7, 115.7, 59.3, 57.9, 54.6, 46.3, 45.9, 31.6, 28.8, 28.4, 27.8, 26.4, 22.9, 22.6, 8.9; MS (APCI+) m/z Calcd (M$^+$): 491.3, Found: 492.3 (M+H$^+$); t_R = 1.55 min (96.1%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-2-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39l).** Compound **24** (0.20 mmol) was coupled to compound **36c** (0.20 mmol) according to general procedure H to yield compound **39l** as a white solid (36 mg, 34%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.29 (1H, s, NH), 7.83 (1H, s, pyrimidine), 7.79 (1H, d, *J* = 6.8Hz, NH), 7.68 (1H, d, *J* = 8.4Hz, Ar), 7.61 (1H, s, Ar), 7.40 (1H, d, *J* = 8.8Hz, Ar), 4.49-4.45 (1H, m, CH(Cp)), 4.18-4.15 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 3.86 (3H, s, O-CH$_3$), 3.80-3.70 (1H, m, NHC$\underline{H}$), 3.21 (3H, s, N-CH$_3$), 2.61 (2H, d, *J* = 13.2Hz, piperidine), 2.15 (3H, s, N-CH$_3$), 2.01-1.48 (16H, m, piperidine, Cp and CHC$\underline{H}_2$CH$_3$), 0.74 (3H, t, *J* = 7.6Hz, CHCH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.2, 163.3, 158.1, 155.3, 152.0, 145.8, 138.9, 131.6, 116.3, 114.3, 110.3, 101.2, 59.4, 58.0, 56.2, 54.3, 46.4, 31.9, 29.3, 29.0, 28.2, 26.9, 23.5, 23.1, 9.5. MS (APCI+) m/z Calcd (M$^+$): 521.3, Found: 522.3 (M+H$^+$); t_R = 1.86 min (97.7%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-fluoro-N-(1-methylpiperidin-4-yl)benzamide **(39m).** Compound **24** (0.20 mmol) was coupled to compound **36d** (0.20 mmol) according to general procedure H to yield compound **39m** as a white solid (35 mg, 34%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.52 (1H, s, pyrimidine), 8.12-8.04 (2H, m, Ar), 7.77 (1H, s, NH), 7.67-7.60 (2H, m, Ar and CONH), 4.27-4.15 (2H, m, CH(Cp) and C$\underline{H}$CH$_2$CH$_3$), 3.69-3.67 (1H, m, NHC$\underline{H}$), 3.20 (3H, s, N-CH$_3$), 2.73 (2H, d, *J* = 11.2Hz, piperidine), 2.13 (3H, s, N-CH$_3$), 1.90-1.48 (16H, m, Cp, piperidine and CHC$\underline{H}_2$CH$_3$),0.72 (3H, t, *J* = 4.8Hz, CHCH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 164.5, 163.4, 155.2, 154.1, 151.9, 138.8, 128.7, 123.7, 121.6, 116.5, 114.5, 114.3, 60.4, 58.9, 54.9, 46.9, 46.3, 31.8, 29.0, 28.8, 28.2, 26.9, 23.6, 23.3, 9.3; MS (APCI+) m/z Calcd (M$^+$): 509.3, Found: 510.3 (M+H$^+$); t_R = 1.55 min 100%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-isobutoxy-N-(1-methylpiperidin-4-yl)benzamide **(39n).** Compound **24** (0.20 mmol) was coupled to compound **36e** (0.20 mmol) according to general procedure H to yield compound **39n** as a white solid (35 mg, 31%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.42 (1H, d, *J* = 8.0Hz, NH), 8.06 (1H, d, *J* = 7.2Hz, Ar), 7.84 (1H, s, pyrimidine), 7.56 (1H, s, NH), 7.49-7.46 (2H, m, Ar), 4.27-4.26 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.10-4.03 (1H, m, CH(Cp)), 3.92 (2H, d, *J* = 6.4Hz, OCH$_2$), 3.80-3.68 (1H, m, NHC$\underline{H}$), 3.24 (3H, s, N-CH$_3$), 2.80 (2H, d, *J* = 10.0Hz, piperidine), 2.19 (3H, s, N-CH$_3$), 2.17-1.58 (17H, m, piperidine, Cp, C$\underline{H}$(CH$_3$)$_2$) and CHC$\underline{H}_2$CH$_3$), 1.05 (6H, d, *J* = 6.0Hz, CH(C$\underline{H}_3$)$_2$), 0.75 (3H, t, *J* = 6.8Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.6, 163.4, 154.5, 151.5, 146.2, 138.4, 132.8, 126.9, 120.8, 116.7, 115.9, 110.5, 75.0, 61.7, 59.9, 54.7, 46.5, 45.7, 31.5, 28.8, 28.4, 28.2, 26.8, 24.3, 19.4, 9.0; MS (APCI+) m/z Calcd (M$^+$): 563.4, Found: 564.4 (M+H$^+$); t_R = 1.57 min (94.8%).

(R)-3-(Benzyloxy)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-N-(1-methylpiperidin-4-yl)benzamide **(39o).** Compound **24** (0.20 mmol) was coupled to compound **36f** (0.20 mmol) according to general procedure H to yield compound **39o** as a white solid (30 mg, 25%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.43 (1H, d, *J* = 8.8Hz, NH), 8.08 (1H, d, *J* = 7.6Hz, Ar), 7.82 (1H, s, pyrimidine), 7.60 (2H, m, Ar and NH), 7.54-7.50 (3H, m, Ar), 7.44-7.35 (3H, m, Ar), 5.25 (2H, s, C$\underline{H}_2$Ph), 4.25 (1H, m, C$\underline{H}$CH$_2$CH$_3$), 4.17-4.12 (1H, m, CH(Cp)), 3.75-3.73 (1H, m, NHC$\underline{H}$), 3.24 (3H, s, N-CH$_3$), 2.80 (2H, d, *J* = 10.0Hz, piperidine), 2.19 (3H, s, N-CH$_3$), 1.96-1.51 (16H, m, piperidine, Cp and CHC$\underline{H}_2$CH$_3$), 0.74 (3H, t, *J* = 6.8Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.5, 163.4, 154.5, 146.1, 138.5, 137.1, 132.9, 128.9, 128.5, 128.2, 127.1, 120.9, 116.6, 116.4, 111.2, 70.8, 61.4, 59.7, 55.0, 46.9, 46.3, 31.9, 28.9, 28.8, 28.2, 26.8, 24.1, 9.1; MS (APCI+) m/z Calcd (M$^+$): 597.3, Found: 598.4 (M+H$^+$); t_R = 1.55 min (100%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-(cyclopentyloxy)-N-(1-methylpiperidin-4-yl)benzamide **(39p).** Compound **24** (0.20 mmol) was coupled to compound **36g** (0.20 mmol) according to general procedure H to yield compound **39p** as a white solid (30 mg, 26%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.43 (1H, d, *J* = 7.6Hz, NH), 8.05 (1H, d, *J* = 8.0Hz, Ar), 7.83 (1H, s, pyrimidine), 7.82-7.46 (3H, m, Ar

and NH), 5.01 (1H, m, OC<u>H</u>), 4.28-4.25 (1H, m, C<u>H</u>CH_2CH_3), 4.18-4.14 (1H, m, CH(Cp)), 3.75-3.72 (1H, m, NHC<u>H</u>), 3.24 (3H, s, N-CH_3), 2.80 (2H, d, J = 10.4Hz, piperidine), 2.19 (3H, s, N-CH_3), 2.03-1.55 (24H, m, piperidine, Cp and CHC<u>H_2</u>CH_3), 0.75 (3H, t, J = 7.2Hz, CH_2C<u>H_3</u>); ^{13}C NMR (DMSO-d_6, 100MHz) δ 165.5, 163.4, 154.4, 151.5, 144.8, 138.5, 133.7, 126.9, 120.7, 116.6, 115.9, 112.4, 80.9, 61.5, 59.8, 55.0, 46.9, 46.3, 32.7, 31.9, 28.9, 28.2, 26.8, 24.3, 23.9, 9.1; MS (APCI+) m/z Calcd (M^+): 575.4, Found: 576.4 ($M+H^+$); t_R = 1.58 min (100%).

(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)oxy)-3-methoxy-N-(1-methylpiperidin-4-yl)benzamide **(39q).** Compound **24** (0.17 mmol) was coupled to compound **38** (0.17 mmol) according to general procedure H to yield compound **39q** as a white solid (20 mg, 22%): ^{1}H NMR (DMSO-d_6, 400MHz) δ 8.22 (1H, d, J = 7.6Hz, NH), 7.76 (1H, s, pyrimidine), 7.56 (1H, s, Ar), 7.51 (1H, d, J = 8.0Hz, Ar), 7.19 (1H, d, J = 8.8Hz, Ar), 4.30-4.20 (1H, m, C<u>H</u>CH_2CH_3), 3.80-3.69 (5H, m, CH(Cp), NHC<u>H</u> and O-CH_3), 3.23 (3H, s, N-CH_3), 2.80 (2H, d, J = 10.4Hz, piperidine), 2.19 (3H, s, N-CH_3), 1.97-1.24 (16H, m, piperidine, Cp and CHC<u>H_2</u>CH_3), 0.71 (3H, t, J = 7.2Hz, CH_2C<u>H_3</u>); ^{13}C NMR (DMSO-d_6, 100MHz) δ 168.5, 165.5, 156.9, 154.9, 150.0, 139.7, 135.5, 130.1, 123.4, 121.1, 112.6, 63.2, 61.7, 56.8, 55.6, 47.6, 47.0, 32.5, 28.9, 28.5, 28.3, 27.5, 24.0, 9.5; MS (APCI+) m/z Calcd (M^+): 522.3, Found: 523.3 ($M+H^+$); t_R = 1.52 min (100%).

In Vitro BRD4 and PLK1 Assays

BROMOscan® and KINOMEscan® K_i measurements were conducted at DiscoveRx (San Diego, CA). BROMOscan and KINOMEscan employ proprietary competition assays directed to ligand binding site to measure interactions between test compounds and bromodomains or kinases. Briefly, T7 phage displaying tandem bromodomains of BRD4 were grown in parallel in 24-well blocks in an *E. coli* host derived from the BL21 strain: *E. coli* were infected with T7 phage and incubated with shaking at 32 °C until lysis (90-150 minutes). The lysates were then centrifuged (5,000 x g) and filtered (0.2μm) to remove cell debris. PLK1 was produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. To generate affinity resins for the assays, streptavidin-coated magnetic beads were treated with biotinylated small molecule or acetylated peptide ligands for 30 minutes at room temperature. The liganded beads were blocked with excess biotin and washed with SEA BLOCK Blocking Buffer (Thermo Fisher, Rockford, IL), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and reduce non-specific phage binding. Binding reactions were assembled by combining DNA-tagged protein, liganded affinity beads, and test compounds in 1x binding buffer (17% SeaBlock, 0.33x PBS, 0.04% Tween 20, 0.02% BSA, 0.004% Sodium azide, 7.4 mM DTT). Test compounds were prepared as 1000x stocks in DMSO and subsequently diluted to ensure a final DMSO concentration of 0.9% for KINOMEscan screens and 0.1% DMSO for BROMOscan screens. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 2 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The DNA-tagged protein concentrations in the eluates were then measured by qPCR. K_i values were obtained using a 3-fold serial dilution across 11 compound concentrations ranging from 0 μM to 10 μM for BROMOscan and 0 μM to 30 μM for KINOMEscan. K_i values were calculated with a standard dose-response curve using Hill equation with a slope of -1. Curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

Cell Viability Assay

A cell viability assay was conducted to obtain GI_{50} values, the compound concentration resulting in 50% growth inhibition, using an acute myeloid leukemia cell line, MV-4-11 (ATCC® CRL-9591™) (ATCC, Manassas, VA). Briefly, cells were seeded on a 96-well flat-bottom cell culture plate at the density of 30,000 cells per well in RPMI supplemented with 10% fetal bovine serum and L-glutamine plus penicillin and streptomycin. MV4-11 cells were maintained at 37 ºC with 5% CO_2 for 24 hours then exposed to a given compound at 11 concentrations ranging from 0 μM to 20 μM in a 150 μL volume in the presence of 0.2% DMSO, the solvent used in serial dilution of the compound. The exposure was maintained for approximately 72 hours and the cell viability was measured using CellTiter-Blue® (Promega, Madison, WI) according to manufacturer's instruction. GI_{50} values were calculated using a four-parameter dose-response model in GraphPad Prism (La Jolla, CA).